Applied and Environmental Microbiology, Nov. 2002, p. 5750–5755 Vol. 68, No. 11
0099-2240/02/$04.00+0 DOI: 10.1128/AEM.68.11.5750–5755.2002

Large-Scale Production of Coenzyme F_{420}-5,6 by Using *Mycobacterium smegmatis*

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Received 7 March 2002/Accepted 30 July 2002

Production of coenzyme F_{420} and its biosynthetic precursor FO was examined with a variety of aerobic actinomycetes to identify an improved source for these materials. Based on fermentation costs, safety, and ease of growth, *Mycobacterium smegmatis* was the best source for F_{420}-5,6. *M. smegmatis* produced 1 to 3 μmol of intracellular F_{420} per liter of culture, which was more than the 0.85 to 1.0 μmol of F_{420}-2 per liter usually obtained with *Methanobacterium thermoautotrophicum* and ~10-fold higher than what was previously reported for the best aerobic actinomycetes. An improved chromatography system using rapidly flowing quaternary aminoethyl ion-exchange material and Florisil was used to more quickly and easily purify F_{420} than with previous methods.

F_{420} is a 7,8-didemethyl-8-hydroxy-5-deazaflavin electron transfer coenzyme first described for methanogenic archaea (10, 11). In the archaea, it is used for a variety of redox reactions that are involved in energy generation (18, 19, 21, 23, 26, 40). F_{420} is used by *Streptomyces* species for tetracycline and lincomycin biosynthesis (7, 28, 35) and may be used in mitomycin C biosynthesis (27). In *Mycobacterium* and *Nocardia* species, F_{420} is used by an F_{420}-dependent glucose-6-phosphate dehydrogenase (32, 33). Photolyases from the green alga *Scenedesmus* species and the cyanobacterium *Synechocystis* species contain bound F_{420} (14, 15). *Methanobacterium thermoautotrophicum* and *Methanococcus voltae* F_{420} contains two glutamates (F_{420}-2) (11, 17), and *Methanosarcina barkeri* contains F_{420} with four and five glutamates (F_{420}-4,5) (17) while *Mycobacterium* spp. contain primarily five- and six-glutamate forms (F_{420}-5,6) (3). FO, a 5-deazaflavin that is a biosynthetic intermediate in the final steps of F_{420} synthesis, is observed in culture supernatants (22, 24, 28, 35). FO is catalytically active in all F_{420}-dependent reactions examined, but its K_m is often higher than that of F_{420} (10, 19, 41). F_{420} is mostly retained inside cells due to the negative charges on the phosphate and glutamyl groups, but the neutral FO more easily leaks out of the cells. The only measurement of the intracellular and extracellular distribution of both FO and F_{420} that we are aware of is that reported by Peck for *Methanosarcina* species (31).

F_{420} is not commercially available, and thus, those who work with this coenzyme must produce and purify it themselves or obtain it from others. Much of the F_{420} used in research is made from *M. thermoautotrophicum*, due to its relatively high F_{420}-2 content per gram of cells and rapid growth rate, although others may use *Methanococcus* or *Methanosarcina* species. Most researchers who study F_{420} have in the past worked with methanogens and thus have been able to grow these organisms. However, as interest has grown in F_{420}-dependent reactions in nonarchaea, scientists not trained in the growth of strictly anaerobic organisms have begun to obtain F_{420} or FO from *Streptomyces* species or related actinomycetes. The general observation has been that with even the best producers ~40-fold-less F_{420} is available from the actinomycetes on a basis of dry weight of cells. About 1.9 μmol of F_{420} per g (dry weight) of cells is present in *M. thermoautotrophicum* (10, 11, 17), whereas the highest yields observed for *Streptomyces* species have been ~0.05 μmol of F_{420} per g (dry weight) of cells (9, 13, 16). Assuming cell densities of 0.5 and 3 g (dry weight) of cells per liter, respectively, for the methanogen and an actinomycete, this predicts yields of 0.95 and 0.15 μmol of F_{420} per liter of culture, respectively. However, one paper has described very high levels of FO in the culture media of some actinomycetes (220 to 550 μmol of FO per liter) (24); unfortunately, F_{420} in the cells or supernatant was not examined. Since the medium used in that study contained very high nutrient levels compared to those for media used in previous studies, we examined F_{420} and FO levels in cells and in culture supernatants of a range of aerobic actinomycetes grown in rich medium with hope of finding an improved source of F_{420}.

Growth of actinomycetes. We obtained cultures of *Actinomadura kijaniata* (ATCC 31588), *Actinoplanes missouriensis* (ATCC 14538), *Streptomyces avermitilis* (ATCC 31267), *Streptomyces flocculus* (ATCC 13257), *Streptomyces coelicolor* (ATCC 19894), *Mycobacterium phlei* (ATCC 11758), *Nocardioides simplex* (ATCC 6946), and *Rhodococcus rhodochrous* (ATCC 13808) from the American Type Culture Collection (Manassas, Va.). *Mycobacterium smegmatis* mc^2 155 was a gift of W. Jacobs, Jr. (Albert Einstein College of Medicine, New York, N.Y.). *Rhodococcus opacus* Rb1 was a gift of R. Blasco (Universidad de Córdoba, Cordoba, Spain). Aerobic actinomycetes were routinely grown in flasks with shaking at 28, 30, or 37°C, depending on their optimal temperature. Inoculum medium contained (in grams per liter) soluble starch (25), glucose (5), soy peptone (10), yeast extract (5), ammonium sulfate (2), and KH_2PO_4 (0.3). MSR production medium contained (in grams per liter) glucose (40), yeast extract (15), soy peptone (15), $NaH_2PO_4 \cdot H_2O$ (1.75), and ferric ammonium citrate (0.04). MSR medium pH was adjusted to 7.0 before

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autoclaving. Flasks were autoclaved with all components present, but for fermentors, glucose was sterilized separately. Cells were grown in inoculum medium for 1 to 3 days. For flask growth, a 10% inoculation was made into MSR medium, which was incubated for 2 to 6 days, until late log or stationary phase. For fermentor growth, a 5% inoculation was made into MSR medium, and cells were grown for about 4 days.

Fermentor growth was conducted in a New Brunswick Scientific Bioflo II 5-liter-working-volume fermentor, with 4 liters of medium. The vessel height was 30 cm, and the internal diameter was 16 cm. The liquid level was 19 cm above the vessel bottom. One Rushton-type six-bladed 7.8-cm-diameter impeller was positioned 7.5 cm from the hemispheric bottom of the vessel. A second impeller was positioned above the liquid surface of the medium, 23.5 cm above the bottom of the vessel; this top impeller helped reduce foam problems. Agitation was initially set at 200 rpm, and the gas rate was set at 1.5 liters/min. After about 20 h of growth, agitation was increased to 400 or 500 rpm. Small amounts (~0.1 ml) of polyethylene glycol 1000 were added several times after the first day of growth to control foam.

HPLC analysis. F_{420} and FO were measured by C_{18} high-pressure liquid chromatography (HPLC) using a fluorescence detector (excitation, 400 nm; emission, 470 nm) (3). Deazaflavin was estimated with a standard curve of known F_{420} concentrations based on the ε_{400} of 25.7 mM^{-1} cm^{-1}. *M. thermoautotrophicum* strain Marburg (grown in a 100-liter fermentor on H_2-CO_2 as described previously [8]) was used as a source of standard F_{420}-2. *M. smegmatis* was used as an F_{420}-5 and F_{420}-6 standard (3). FO purified from the supernatant of *M. smegmatis* was used as a routine FO standard, the identity of which was confirmed by coelution with synthetic FO (2). For analytical purposes, supernatant was separated from cells by centrifugation of culture samples at 3,500 × *g* for 10 min, followed by decanting of the supernatant into another tube and frozen storage until direct analysis by appropriate dilution and HPLC. Cells (from 10 or 35 ml of culture) were extracted twice by being boiled in 1 ml of 25 mM Na acetate (pH 4.7) for 15 min, followed by centrifugation, and the two supernatants were combined, diluted, and analyzed. Pellets from this extraction were dried at 95°C for >2 days and weighed to determine dry weights. Extraction with 50 to 70% ethanol without boiling was not as effective.

Predominant F_{420} forms made by aerobic actinomycetes. HPLC analysis of the actinomycete cell extracts revealed two major peaks which corresponded to F_{420}-5 and F_{420}-6 from *M. smegmatis*. Very little F_{420}-2 was present. Although earlier work had presented evidence that the F_{420} in some *Streptomyces* species had more than two glutamyl groups (based on thin-layer chromatography or thin-layer electrophoresis data [12, 25]), our analysis provides good evidence that specifically the F_{420}-5 and F_{420}-6 forms predominate in many aerobic actinomycetes. FO was also identified in cells and the medium.

Production levels of F_{420} and FO in flask experiments. The best producers of F_{420} in flasks were *S. flocculus*, *M. smegmatis*, and *M. phlei*, and most actinomycetes examined contained ~0.1 to 0.6 μmol per g (dry weight) of cells (Table 1). FO accounted for about 2 to 10% of the cellular deazaflavin. FO generally predominated in the supernatant and represented a significant amount of total deazaflavin for potential recovery. F_{420} yields here were higher than those that we previously reported for several actinomycetes grown in weaker medium (~0.02 to 0.06 μmol per g [dry weight] of cells) (9). These high yields could be due to the increased medium strength in the present work (two- and threefold-higher levels of carbohydrate and peptone, respectively, plus other nutrients, compared to medium that we had previously used [9]). The *M. thermoautotrophicum* cellular F_{420} yield in term of micromoles per liter was 1.5- to 5-fold lower than those of *M. smegmatis* and *S. flocculus*, although on a per-gram basis the methanogen was higher; this reflects the much higher actinomycete cell densities (Table 2). Volumetric production was highest with *S. flocculus*. Volumetric production by flask-grown *M. smegmatis* was 7.5- to 23-fold higher than the levels produced by *S. coelicolor* and *Streptomyces griseus*, which until the present work had been the best F_{420}-producing actinomycetes (Table 1).

Due to the very high FO levels reported in the medium of actinomycetes by Kuo et al. (24) and the fact that we did not see such levels in our work (Table 1), we were concerned that a special medium was required to obtain such high FO levels. Thus, we examined F_{420} and FO production by *S. avermitilis*, *A. kijaniata*, *M. smegmatis*, and *A. missouriensis* in a molasses-cottonseed medium that was essentially identical to their medium and which contained (in grams per liter) glucose (15), blackstrap molasses (20), soluble starch (40), cottonseed hydrolysate (25), $CaCO_3$ (8), and K_2SO_4 (2). In our initial experiments, for F_{420} and FO estimation we used quaternary aminoethyl (QAE) ion-exchange chromatography followed by conventional bench top non-HPLC C_{18} chromatography of cell extract and supernatant samples while monitoring the effluent with spectrofluorimetry, followed by deazaflavin measurement by UV-visible light spectral analysis, not by HPLC. The molasses-cottonseed medium was problematic because much dark material bound permanently to the QAE column material, limiting QAE recycle options, but repeatable 5-deazaflavin estimates were obtained. We varied growth temperature and agitation speed and took samples during growth of several of the higher-yielding species (data not shown). In all cases, using this partial purification and absorbance measurement approach, we observed less than 1 μmol of FO per liter (data not shown), which was significantly less than that reported by Kuo et al. (24). We then used HPLC without prior chromatography to directly examine cultures of *S. flocculus*, *S. avermitilis*, and *M. smegmatis* grown on molasses-cottonseed medium. These yielded, respectively, 1.7, 1.5, and 2.1 μmol of F_{420} per liter, and FO levels in the spent medium ranged from not detectable to 0.75 μmol/liter. We conclude that similar results were obtained with both of our methods but that the lower values that arose from the absorbance approach were due to both chromatography processing losses and the inherent low sensitivity of absorbance and that the higher values resulting from HPLC are a result of the higher specificity of fluorescence and little loss from chromatographic steps, since the sample was only diluted and injected. Also, our HPLC measurements with *M. thermoautotrophicum* (Table 1) are consistent with yields reported by several other laboratories using different methods (10, 11, 17), giving us confidence in our methodology.

We could not find conditions that led to production of the very high FO levels (>200 μmol/liter) reported previously (24). The differences between our results and those of Kuo et

TABLE 1. F_{420} and FO levels in cells and culture supernatants[a]

Organism and reference(s)	Level in cells				Level in supernatant			
	F_{420}		FO		F_{420}		FO	
	μmol/g	μmol/liter	μmol/g	μmol/liter	μmol/g	μmol/liter	μmol/g	μmol/liter
S. flocculus								
This work	0.62	4.43	0.056	0.515	0.37	2.95	0.28	2.58
Ref. 9	0.023	0.069	NR[b]	NR	NR	NR	NR	NR
M. smegmatis	0.30	1.43	0.020	0.103	0.04	0.20	0.25	1.24
M. phlei	0.17	0.95	0.003	0.017	0.04	0.24	0.15	0.83
A. kijaniata	0.17	0.78	0.006	0.025	0.20	0.95	0.05	0.23
S. avermitilis	0.16	0.59	0.010	0.034	0.10	0.28	0.15	0.45
R. opacus Rb1	0.19	0.46	0.020	0.045	0.09	0.19	0.81	1.75
A. missouriensis	0.19	0.25	0.021	0.027	0	0	0.02	0.01
R. rhodochrous	0.11	0.20	0.008	0.150	0	0	0.55	1.00
N. simplex	0.08	0.15	0.006	0.012	0	0	0.47	0.90
S. coelicolor								
This work	0.04	0.12	0.003	0.095	0.38	1.15	0.10	0.33
Ref. 13	0.06	0.19	NR	NR	NR	NR	NR	NR
S. griseus								
Ref. 9	0.021	0.063	NR	NR	NR	NR	NR	NR
Ref. 16	0.058	0.17	NR	NR	NR	NR	NR	NR
M. thermoautotrophicum								
This work[c]	1.7	0.85	0.03	0.02	ND[d]	ND	ND	7.2[e]
Refs. 10, 11[f]	2.0	1.0	NR	NR	NR	NR	NR	8.3[g]
Methanosarcina barkeri								
Refs. 8, 17[h]	0.76	2.6	NR	NR	NR	NR	NR	NR
M. formicicum								
Refs. 17, 36[i]	1.2	0.60	NR	NR	NR	NR	NR	NR
Methanococcus, voltae								
Refs. 17, 36[i]	0.034	0.017	NR	NR	NR	NR	NR	NR
Refs. 8, 17[j]	0.15	0.073	NR	NR	NR	NR	NR	NR

[a] For growth in flasks, unless noted otherwise. Deazaflavin levels are expressed as micromoles per gram (dry weight) of cells or micromoles per liter of culture. Ref., reference.
[b] NR, not reported.
[c] Cells were fermentor grown in this laboratory, and 5-deazaflavin levels were measured by HPLC.
[d] ND, not determined.
[e] From reference 22.
[f] Values are averages of two determinations by Eirich et al. (10, 11) with fermentor-grown cells, assuming that the dry weight of cells equals 1/7 the wet weight of cells and assuming a culture density of 0.5 g (dry weight) of cells per liter. One determination was made by absorbance of purified material, and the other was made by enzyme assay.
[g] From references 8, 17, and 34.
[h] Grown on methanol; volumetric production was estimated by assuming a yield of 1.7 g (dry weight) of cells per liter in a fed bottle.
[i] Grown on formate in bottles; volumetric production was estimated for a pH-controlled fermentor by assuming a yield of 0.5 g (dry weight) of cells per liter.
[j] Grown on H_2 plus CO_2 in bottles; volumetric production was estimated by assuming a fermentor yield of 0.5 g (dry weight) of cells per liter.

TABLE 2. Comparison of factors involved in choosing an organism for producing F_{420}

Organism	Ease of growth	Time for growth (days)	Hazards	Approx cell yield (g [dry, wt] of cells/liter)	Estimated fermentation cost ($) per[a]:			
					g (dry, wt) of cells	Liter of culture	μmol of F_{420}/cell	μmol of FO/supernatant
Methanobacterium thermoautotrophicum	Difficult	3–5; variable	Flammable, explosive gas	0.5	5.0	25	27	3.2
Streptomyces flocculus	Easy	3–4	No infections; produces streptonigrin, a toxic antitumor drug	7.2	2.1	15	3.4	5.8
Mycobacterium smegmatis	Easy	2–4	Chance of wound infection; no toxin	4.8	3.1	15	7.5	7.5
Mycobacterium phlei	Easy	2–4	Chance of wound infection; no toxin	5.6	2.7	15	7.5	7.5

[a] Based on data in Table 1 for *S. flocculus* and *M. thermoautotrophicum* and on fermentor data for *M. smegmatis* and *M. phlei* (2 μmol each of F_{420} and FO per liter). Data are based on our mid-2000 estimates at the University of Iowa for the growth of *M. thermoautotrophicum* ($2,000/100 liters) and *Streptomyces* ($1,500/100 liters) and an estimate in early 2001 from Eric Johnson at the University of Illinois for *M. thermoautotrophicum* ($600/20 liters). Estimates are for the production of a wet cell pellet, thus incorporating the costs of medium components, fermentor growth, and cell harvest, but not the costs for cell extraction and deazaflavin purification. The methanogen estimates were averaged. It should be noted that university-based fermentor facilities are typically subsidized to some degree, and thus, the costs given here are probably lower than the true costs.

al. do not arise from strain differences, since at least in the case of *A. kijaniata* and *S. avermitilis* the same American Type Culture Collection strains were used (D. A. Yurek, personal communication). We do not know the reason for the difference between our data and those reported previously (24), but we noted that the molasses-cottonseed medium showed several large HPLC peaks which eluted near FO, and that may interfere with HPLC analysis. The lack of agreement concerning the quantities of 5-deazaflavin produced in our study and the previous study does not diminish the importance of the two major conclusions of Kuo et al. that 5-deazaflavin is required for lincomycin production and that many aerobic actinomycetes produce 5-deazaflavin (7, 24). Furthermore, the earlier work led us to try a much stronger medium, which allowed us to produce levels of F_{420} per liter that are >10-fold higher than previously reported for the best-producing actinomycetes.

We conclude that *S. flocculus*, *M. smegmatis*, and *M. phlei* produce more cellular F_{420} and more supernatant FO than are produced by the four strains previously described as very high FO producers (*A. kijaniata*, *S. avermitilis*, *A. missouriensis*, and *S. coelicolor*). These findings are important for those wishing to make F_{420} for their own use, because it corrects the impression that ~100 μmol of F_{420} per liter might be produced by these organisms and identifies several organisms which produce ~2 μmol of F_{420} per liter and which can serve as good sources of F_{420} compared to the methanogens and most aerobic actinomycetes.

F_{420} production in a fermentor. We examined deazaflavin production by two of our best F_{420} producers in a fermentor. After 70 to 90 h, six separate fermentor runs with *M. smegmatis* made 1 to 3 μmol of cellular F_{420} per liter and 1 to 6 μmol of FO per liter of supernatant. Little 5-deazaflavin was present before 50 h. Impeller speed (400 versus 500 rpm) did not affect yield. Two fermentor runs with *M. phlei* gave similar results but created more wall growth than with *M. smegmatis*, making *M. phlei* cultures more difficult to monitor and harvest.

Identification of the best organisms for F_{420} production. Based on volumetric F_{420} production (micromoles per liter of culture) and on several factors listed in Table 2, the best actinomycetes are more attractive than methanogens for F_{420} production, if it is not important to have F_{420}-2 instead of F_{420}-5,6. Growth of methanogens requires extensive experience with anaerobic technique and requires a variety of tools for the task, many of which are not commercially available (8, 37). *M. thermoautotrophicum* is grown in a fermentor that contains pressurized hydrogen gas, which along with the methane produced creates a flammability hazard. Most fermentors require modification prior to use for this purpose. *Methanosarcina barkeri*, *Methanobacterium formicicum*, and *Methanococcus* species (e.g., *Methanococcus voltae* or *Methanococcus vannielii*) can be grown without the use of hydrogen gas, with the use instead of methanol or formate as growth substrate and nitrogen gas to maintain anaerobic conditions (8, 36, 37). While this simplifies growth of these methanogens, specialized equipment and experience with anaerobic growth are still required. *Methanosarcina barkeri* in particular is prone to contamination (although this can be avoided by appropriate use of antibiotics), and obtaining colonies of any methanogen on petri plates requires a great deal of experience and generally is done with an anaerobic glove bag and anaerobic jar. In contrast, aerobic actinomycetes are easy to grow, and their growth period is more predictable. The cell yields of the better actinomycetes are about 10-fold higher than those of the methanogens. This offsets the lower actinomycete internal F_{420} yields per gram (dry weight) of cells, since from a cost perspective the yield of F_{420} per liter of fermentor broth is the most important under most fermentation facility cost structures. Nonetheless, for kinetic reasons, work with a methanogen enzyme may be best done with F_{420}-2, rather than the mix of F_{420}-5 and F_{420}-6 that predominates in *Mycobacterium* species. Also, if appropriate skilled personnel are available in a financially subsidized facility, F_{420}-2 purification from a methanogen would require processing much less cell mass and would thus save on the costs of purification that have not been estimated here. Thus, in these instances, it is worth exploring F_{420} production by a methanogen grown on formate in a pH-controlled fermentor (20, 36) and the more traditional use of *M. thermoautotrophicum* (8).

Of the top five F_{420} producers in Table 1, we are most confident in the safety of working on a large scale with the two *Mycobacterium* species. *S. flocculus* presents no infection hazard, but some strains produce streptonigrin, a toxic antitumor antibiotic, which could be a hazard (4). A similar objection could be made to using *A. kijaniata* and *S. avermitilis*, which produce the toxic compounds kijanimicin and avermectin, respectively (5, 6). Thus, we prefer to not use these organisms for F_{420} production. *M. smegmatis* is not normally considered pathogenic, but more than 20 human infections with this organism have been documented, mostly due to accidental or surgical trauma (29, 39, 42). Two cases of aspiration pneumonia have been reported, but the patients already had serious pulmonary disease. Only three instances of *M. phlei* infections have been reported, one each in the foot, knee, and peritoneal cavity (1, 30, 38). Since fermentor growth involves the risk of scraping and puncture wounds, when *M. smegmatis* and *M. phlei* are being grown we recommend that latex or plastic gloves be worn when working with the system and that puncture-resistant leather gloves be used when inoculating the culture by needle. All wounds that may have introduced mycobacteria should be cleaned thoroughly, and if appropriate (e.g., for penetrating wounds), medical attention should be sought. These precautions are no greater than those required for safe growth of a conventional *Escherichia coli* strain.

As shown in Table 2, consideration of fermentor costs, and cellular yield per liter, leads to estimates of \$27/μmol of F_{420} with *M. thermoautotrophicum* and \$3.40 to 7.50 with *S. flocculus*, *M. smegmatis*, or *M. phlei*. Although FO is catalytically active, and it may be useful for some experiments, most researchers need F_{420} more than FO. This makes the cost per micromole of F_{420} the most important consideration for most situations. Thus, based on ease of growth, fewer hazards, and lower costs, *M. smegmatis* is the best source for F_{420} out of the organisms that we have examined.

Purification of F_{420} from actinomycetes. A procedure for purifying F_{420} from cells was developed by using Macro-Prep High Q QAE (Bio-Rad) and Florisil (Sigma; F-9127) for column chromatography. As an example, fermentor-grown *M. smegmatis* cells (600 g [wet weight]), which based on HPLC analysis contained 44.2 μmol of F_{420} and 7.5 μmol of FO, were processed by this procedure. Cells were mixed with 600 ml of

25 mM $NaPO_4$ buffer (pH 7.0), autoclaved at 121°C for 10 min, and centrifuged. The procedure was repeated three more times (but with 450 ml of buffer), and the pooled supernatant was adjusted to pH 7.0. The supernatant (2.1 liters) was applied to a QAE column (5 by 20 cm) that had been equilibrated with 25 mM $NaPO_4$ (pH 7.0), and the column was washed with 2 liters of 100 mM NaCl in the buffer. The FO was eluted with 4.0 liters of 250 mM NaCl in buffer, and F_{420} was eluted in four 2.0-liter volumes of 400 mM NaCl in buffer. QAE chromatography yielded 34.8 μmol of F_{420} (79% recovery, by HPLC). Florisil was cleaned with 6 N HCl, water, and acetone and then dried at 90°C prior to use. The F_{420} fractions (8 liters) were pooled and adjusted to pH 4.7, then applied to a 5- by 20-cm Florisil column equilibrated with 25 mM Na acetate (pH 4.5), and washed with 2 liters of 400 mM NaCl in buffer. The F_{420} was eluted by a series of 2.0-liter solutions: 100 mM NaCl in buffer, Na acetate buffer alone, water at pH 4.7, and finally twice with water at pH 7. F_{420} (31.5 μmol, by HPLC) eluted in the pH 7 water (95% recovery for the column). Material from this large-scale preparation still contained impurities as indicated by its slightly browner color than that of pure F_{420}, but after rotary evaporation to 500 ml, HPLC indicated that it was about 70% F_{420}-5,6 and 25% F_{420}-2,3,4, with no other significant fluorescent peaks. Omission of the wash or elution steps prior to pH 7 water reduced the purity of the final product, and reduction of the pH 7 water volume reduced the yield. The F_{420} produced by this method can be used effectively for routine enzyme assays and other purposes, but it can then be purified further by gradient chromatography at a more convenient scale, and by HPLC, as needed. The High Q column material had an improved flow rate and good binding capacity compared to the more conventional QAE Sepharose or Sephadex. The Florisil was more efficient at binding the deazaflavins and had a better flow rate than that of the C_{18} material that we have used in the past (32). It is likely that FO purification from a fermentor supernatant by similar QAE and Florisil techniques would also have advantages over previously used methods.

ACKNOWLEDGMENTS

We thank William R. Jacobs, Jr. (Albert Einstein College of Medicine), for providing us with a culture of *M. smegmatis* mc^2 155, R. Blasco (Universidad de Córdoba) for giving us a culture of *R. opacus* Rb1, and David Gibson (University of Iowa) for use of his fermentor.

This work was supported by National Institutes of Health grant GM56177 and U.S. Department of Agriculture grant 4132008 to L.D.

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